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# Effect of prostaglandin E in multiple experimental models. VII. Effect on resistance to sepsis*



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*The immunosuppression seen following burn injury has frequently been attributed to elevated prostaglandin E levels. We evaluated the contribution of elevated prostaglandin E levels on susceptibility to infectious complications utilizing multiple mouse models. The administration of 100 μg/kg of the long-acting derivative of prostaglandin E, 16,16-dimethyl-prostaglandin E, was found to improve survival in C3/HEN mice challenged with $1 \times 10^8$ Escherichia coli organisms intraperitoneally. The administration of indomethacin was found to decrease survival in the same model. With C3/HEJ (endotoxin-resistant) mice, indomethacin was found to increase mortality rates in animals challenged with $1 \times 10^8$, $1 \times 10^9$ or $1 \times 10^{10}$ Escherichia coli organisms. These findings suggest that elevated prostaglandin E levels seen in burn patients may not be responsible for the postburn increased susceptibility to infectious complications.*

## Introduction

One of the primary aetiologies for mortality following burn injury is the development of infectious complications (Sevitt, 1979). These infections have two main causes, a loss of the skin's natural barrier and the immunosuppression that results from burn injuries. This immunosuppression is due to a number of factors, including inadequate nutrition in the postburn period, the use of immunosuppressive agents such as anaesthesia and blood transfusions, and the release of endogenous immunosuppressive metabolites.

Prostaglandin E (PGE) has been reported to be one of the immunosuppressive metabolites released following burn injury (Ninneman and Stockland, 1984). The belief in an immunosuppressive nature of PGE has resulted from two areas of investigation. The first is the demonstration by Arturson (1976) that PGE levels are increased following burn injury and that burn patients are immunosuppressed (Warden, 1986). The second is the demonstration that PGE impairs immune function in a number of *in vitro* leucocyte culture models (Faist et al., 1987).

There has been little investigation on the *in vivo* effects of PGE in animal models due to the extremely short half-life of parenterally administered PGE (Jaffe et al., 1988). The short half-life is the result of the near total clearance of PGE during each pass through the lungs. Derivatives of PGE that are resistant to enzymic degradation by the pulmonary parenchyma and thus have much longer half-lives have recently been developed. One of these derivatives is 16,16-dimethyl-prostaglandin E (dPGE). We herein report an evaluation of this agent on resistance to sepsis in multiple mouse models.

## Materials and methods

### Animals

Five hundred adult male C3/HEJ mice and 210 adult male C3/HEN mice weighing approximately 25 g were used in these studies. The mice were housed in stainless-steel hanging cages and allowed food and water *ad libitum*. The mice were observed for a minimum of 1 week prior to entry into the study to exclude the presence of pre-existing diseases.

### Drugs

The dPGE was generously supplied by the Upjohn Company (Kalamazoo, MI, USA) The dPGE was diluted with sufficient normal saline to achieve a concentration that permitted the desired dose of drug to be administered in a final volume of 0.25 ml. The dPGE was injected intraperitoneally through a 25-gauge needle.

Indomethacin was generously supplied by Merck Sharp & Dohme (Columbus, OH, USA). The indomethacin was also diluted with sufficient normal saline to achieve a final concentration that permitted the desired dose to be administered in a final volume of 0.25 ml. The indomethacin was injected intraperitoneally through a 25-gauge needle.

### Sepsis models

Six sepsis models were chosen, all of which utilized intraperitoneal injections of varying quantities of *Escherichia coli* organisms. The *E. coli* were cultured in trypticase soy broth at 37°C for 16 h and then centrifuged at 3000 r.p.m. for 5 min. The supernatant was decanted and the *E. coli* pellet resuspended in a sufficient volume of saline to achieve the desired concentration of organisms. For the C3/HEN mice, two quantities of the *E. coli* were tested. For the first, $1 \times 10^7$ c.f.u. of the *E. coli* organisms were given intraperitoneally in a

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The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other Federal statutes and regulations relating to animals and studies involving animals and with the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health Publication 86–23.


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volume of 0.5 ml saline. For the second, $1 \times 10^8$ c.f.u. of the *E. coli* were given in 0.5 ml of saline.

The *E. coli* organisms were administered at four concentrations to the C3/HEJ mice. For the first, $1 \times 10^7$ c.f.u. in 0.5 ml of saline were given. The second was $1 \times 10^8$ c.f.u. in 0.5 ml of saline. For the third, $1 \times 10^9$ c.f.u. in 0.5 ml of saline were given. The final concentration was $1 \times 10^{10}$ c.f.u. in 0.5 ml of saline.

In each of the peritonitis models, the mice were randomized to one of four drug treatment groups. The first received twice daily injections of 0.25 ml of normal saline intraperitoneally. The second received twice daily intraperitoneal injections of 4 mg/kg of indomethacin dissolved in 0.25 ml of saline. The third group received twice daily injections of 50 μg/kg of dPGE dissolved in 0.25 ml of saline intraperitoneally and the final group received twice daily injections of 100 μg/kg of dPGE dissolved in 0.25 ml of saline intraperitoneally. *Table I* lists the number of animals in each drug treatment group for each of the models.

With each model, the mice were followed for 7 days after peritoneal challenge to determine mean survival times and absolute survival rates. Those mice surviving to 7 days had previously been noted to have no further mortalities. For the calculation of mean survival times, the mice which survived 7 days were given a survival time of 7 days.

All data are presented as mean ± s.e.m. Comparisons among groups were performed using chi-square and Kruskal–Wallis tests.

## Results

The C3/HEN mice challenged with $1 \times 10^7$ *E. coli* c.f.u. had a 100 per cent survival rate in the saline control group, the 50 μg/kg dPGE treatment group and the 100 μg/kg dPGE treatment group (*Table II*). Those mice receiving indomethacin had a decreased survival rate of 16 per cent ($P < 0.001$). The mean survival time for the indomethacin-treated mice was also significantly decreased when compared with the other groups ($P < 0.0001$) (*Table II*).

For C3/HEN mice challenged with $1 \times 10^8$ *E. coli* c.f.u., the survival rates of the saline-treated and 50 μg/kg dPGE-treated groups were 8 per cent (*Table II*). The indomethacin treatment group had a 0 per cent survival rate and the mice treated with 100 μg/kg of dPGE had a survival rate of 32 per cent. These differences were statistically significant ($P < 0.01$). The increased mean survival time of the mice treated with 100 μg/kg of dPGE (*Table II*) was also statistically significant when compared with the other groups ($P < 0.005$).

The C3/HEJ mice injected with $1 \times 10^7$ *E. coli* c.f.u. had a 94 per cent survival rate in the saline treatment group (*Table III*). Both the 50 μg/kg and the 100 μg/kg dPGE treatment groups had an 84 per cent survival rate. The indomethacin-treated mice had a 76 per cent survival rate. These differences were not statistically significant. The differences in the mean survival times among these four groups were also not statistically significant (*Table III*).

**Table I.** Number of mice used for each sepsis model (represented by colonies of *E. coli*) in each treatment group

| | *C3/HEN Mice* | | *C3/HEJ Mice* | | | |
|---|---|---|---|---|---|---|
| *Treatment group* | *$1 \times 10^7$* | *$1 \times 10^8$* | *$1 \times 10^7$* | *$1 \times 10^8$* | *$1 \times 10^9$* | *$1 \times 10^{10}$* |
| Saline | 35 | 25 | 50 | 25 | 50 | 25 |
| Indomethacin | 25 | 25 | 25 | 25 | 25 | 25 |
| 50 μg/kg dPGE | 25 | 25 | 25 | 25 | 50 | 25 |
| 100 μg/kg dPGE | 25 | 25 | 25 | 25 | 50 | 25 |

**Table II.** Mean survival times (mean ± s.e.m.) and survival rates in C3/HEN mice challenged with $1 \times 10^7$ or $1 \times 10^8$ colonies of *E. coli*

| | *$1 \times 10^7$* | | *$1 \times 10^8$* | |
|---|---|---|---|---|
| *Treatment group* | *Mean survival time (days)***** | *Survival rate (%)*** | *Mean survival time (days)**** | *Survival rate (%)** |
| Saline | 7.00 ± 0.00 | 100 | 1.52 ± 0.33 | 8 |
| Indomethacin | 2.88 ± 0.44 | 16 | 1.16 ± 0.13 | 0 |
| 50 μg/kg dPGE | 7.00 ± 0.00 | 100 | 1.52 ± 0.33 | 8 |
| 100 μg/kg dPGE | 7.00 ± 0.00 | 100 | 2.92 ± 0.57 | 32 |

**** $P < 0.0001$; ** $P < 0.001$; *** $P < 0.005$; * $P < 0.01$.

**Table III.** Mean survival times (mean ± s.e.m.) and survival rates of C3/HEJ mice challenged with $1 \times 10^7$, $1 \times 10^8$, $1 \times 10^9$ and $1 \times 10^{10}$ colonies of *E. coli*

| | *$1 \times 10^7$* | | *$1 \times 10^8$* | | *$1 \times 10^9$* | | *$1 \times 10^{10}$* | |
|---|---|---|---|---|---|---|---|---|
| | *Mean survival time (days)* | *Survival rate (%)* | *Mean survival time (days)*** | *Survival rate (%)** | *Mean survival time (days)*** | *Survival rate (%)** | *Mean survival time (days)*** | *Survival rate (%)** |
| Saline | 6.72 ± 0.16 | 94 | 7.00 ± 0.00 | 100 | 6.20 ± 0.38 | 92 | 6.84 ± 0.16 | 96 |
| Indomethacin | 5.76 ± 0.46 | 76 | 4.64 ± 0.45 | 44 | 3.28 ± 0.26 | 4 | 3.68 ± 0.40 | 16 |
| 50 μg/kg dPGE | 6.20 ± 0.38 | 84 | 7.00 ± 0.00 | 100 | 7.00 ± 0.00 | 100 | 7.00 ± 0.00 | 100 |
| 100 μg/kg dPGE | 6.25 ± 0.25 | 84 | 7.00 ± 0.00 | 100 | 6.80 ± 0.14 | 96 | 7.00 ± 0.00 | 100 |

* $P < 0.001$; ** $P < 0.0001$.

For C3/HEJ mice challenged with $1 \times 10^6$ *E. coli* c.f.u., the indomethacin treatments significantly decreased both survival rates and mean survival times (*Table III*). The 44 per cent survival rate was statistically significant ($P < 0.001$), as was the $4.64 \pm 0.45$ day mean survival time ($P < 0.0001$) when compared with the saline and dPGE treatment groups.

The detrimental effect of indomethacin treatment was also demonstrated in the C3/HEJ mice challenged with $1 \times 10^9$ *E. coli* c.f.u. (*Table III*). Both the 4 per cent survival rate and the $3.28 \pm 0.26$ day mean survival time were statistically significant when compared with the other three groups ($P < 0.001$ for survival rates and $P < 0.0001$ for mean survival times).

Finally, at the $1 \times 10^{10}$ *E. coli* c.f.u. challenge, indomethacin treatment adversely affected survival in the C3/HEJ mice. With this quantity of bacterial challenge, the indomethacin-treated mice had a 16 per cent survival rate and a $3.68 \pm 0.40$ day mean survival time, which were both statistically significant when compared with remaining groups ($P < 0.001$ for survival rates and $P < 0.0001$ for mean survival times).

## Discussion

Infection remains a major cause of morbidity and mortality following thermal injuries. The immunosuppression seen following burns is one of the main reasons for this elevated infection rate.

Elevated PGE levels have long been thought to be a contributing factor to the immunosuppression seen in burn patients because of the high levels demonstrated in burn patient serum and because of its toxic effects on leucocyte function in studies *in vitro*.

Attemps to quantitate the contribution of PGE to the postburn immunosuppression have been thwarted by the extremely short half-life of parenterally administered PGE. To avoid this limitation, we have utilized a long-acting derivative of PGE (dPGE) to quantitate the contribution of elevated PGE levels to susceptibility to infection-related mortality.

We have previously reported that the administration of dPGE to Lewis rats increased survival rates in an *E. coli* peritonitis model (Waymack and Yurt, 1988). This beneficial effect appears due, at least in part, to an increased resistance to endotoxin shock (Waymack et al., submitted (a)) when treatment with dPGE commenced prior to endotoxin challenge. When the administration of dPGE was delayed until after endotoxin challenge, the protective effect was no longer apparent. It was further shown that pretreatment with the PGE synthesis inhibitor indomethacin decreased survival rates in Lewis rats challenged with endotoxin (Waymack et al., submitted (b)). The protective effect of dPGE in endotoxin shock appears due, at least in part, to its ability to decrease the rate of release of tumours necrosis factor (Waymack et al., submitted (a)). Finally, the administration of dPGE appears to exert a beneficial effect in septic rats by triggering an amino acid flux from skeletal muscle protein to acute phase proteins (Waymack et al., 1989; Waymack and Mason, 1989).

Our current studies have attempted to determine whether the beneficial effect of dPGE administration is a species-specific trait of rats. To answer this question, two strains of mice were utilized. The first group, C3/HEN mice, are endotoxin sensitive and the second group, C3/HEJ mice, are endotoxin resistant due to their inability to synthesize tumour necrosis factor in response to endotoxin exposure (Beutler et al., 1986).

The results with C3/HEN (endotoxin-sensitive) mice demonstrated a protective effect of elevated PGE levels as evidenced by an increased survival in those animals receiving the dPGE and decreased survival among those receiving indomethacin. These findings strongly suggest that the previously demonstrated beneficial effect of dPGE administration in septic rats is not a species-specific response.

In C3/HEJ (endotoxin-resistant) mice, there was no significant mortality in the saline treatment groups, even at the $1 \times 10^{10}$ c.f.u. of *E. coli* challenge. The ability of this strain of mouse to resist such a high concentration of *E. coli* organisms is probably due, at least in part, to its inability to synthesize the highly toxic macrophage metabolite tumour necrosis factor following the endotoxin exposure. As such, this prevented any possible demonstration of a beneficial effect of dPGE administration.

It was, however, noteworthy that indomethacin treatment of the C3/HEJ mice did result in a significant decrease in survival in three of the *E. coli* peritonitis models. Since C3/HEJ mice are not capable of synthesizing tumour necrosis factor, the detrimental effect of indomethacin cannot be attributed to the PGE/tumour necrosis factor interaction which has previously been demonstrated in rats (Waymack et al., submitted (a)). The increased mortality rate in the indomethacin-treated mice may be due to the effect of indomethacin on blood flow to various organs, on release of other toxic compounds of leucocytes in response to bacterial endotoxin exposure, or on the inhibition of the normal physiological response to sepsis. Such an inhibition has previously been demonstrated in burned septic rats treated with the cyclo-oxygenase inhibitor ibuprofen (Waymack, 1989). The rats which received ibuprofen in that study failed to show the normal hypermetabolic response to sepsis and had a significantly higher mortality rate when compared to the rats treated with saline. Fink et al. (1984) reported that the use of cyclo-oxygenase inhibitors prevented the normal hyperdynamic response in septic dogs. When one considers the increased physiological workload septic patients must accomplish, the benefits of preventing a hyperdynamic/hypermetabolic state must be questioned. Further studies will be required to determine the contribution of these factors to the increased mortality rate.

In conclusion, the elevation of PGE levels seen in burn patients may be a normal physiological response of the body to protect against infection-related mortality which is so common in burn patients. Attempts to decrease the rate of PGE synthesis in the burn patient through the use of cyclo-oxygenase inhibitors may only increase the mortality rate in those patients who develop infections. Further studies delineating the physiological importance of PGE in sepsis should be undertaken prior to initiation of trials using cyclo-oxygenase inhibitors in septic patients.

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## Tanner–Vandeput Prize for Burn Research 1990 Award

The 1990 Tanner–Vandeput Prize for Burn Research, consisting of a cash payment, will be awarded at the 8th International Congress on Burn Injuries of the ISBI, to be held November 11–16, 1990 in New Delhi, India. The Prize will go to a person or person who, in the opinion of the Prize Committee, has made a substantial and outstanding contribution to any aspect of the burn field in their lifetime (i.e., a 'senior investigator's' award). The recipient does not have to be a member of the ISBI or a physician, but be responsible for a major advancement in the treatment of burns.

Nominations for the 1990 Prize may be made by colleagues of those who have made such a contribution to burn care in their lifetime. A candidate may also make an application on his own behalf.

Anyone interested in applying for the 1990 Tanner–Vandeput Prize for Burn Research should send the following information to the ISBI Secretary-General at the address below.

Information required to apply for the Tanner–Vandeput Prize for 1990:

- *Letter of nomination (can be sent by candidate or by someone else)*
- *Description of work, including samples and documentation*
- *Current Curriculum Vitae*
- *Letters of support from colleagues*

Send five copies of this information to:

**Dr. John A. Boswick,**
**International Society for Burn Injuries,**
**2005 Franklin St. #660,**
**Denver, Colorado 80205, USA. Tel (303) 839–1694.**

Deadline for receipt of applications: July 31, 1990

### Information regarding the Tanner–Vandeput prize for burn research

The Prize was established in 1984 by Dr J. C. Tanner of Atlanta, Georgia, co-inventor with Dr. Jacques Vandeput of the Tanner–Vandeput Mesh Dermatome. This Prize was conceived and established to promote the aims of the International Society for Burn Injuries and to motivate individual investigators to do research, study, undertake patient care and treatment and other aspects of the burns problem, and will be awarded to one who has made a substantial contribution to burn care in their lifetime (a 'senior investigator's' award). The Prize consists of a cash payment.

A foundation was created for the sole purpose of awarding the Prize every four years and has separate funds invested to produce income used for the Prize. A trust fund is owned by the 'International Burn Foundation of the United States,' an organization entirely separate from the ISBI. The funds do not overlap or mingle in any way with those of the ISBI.

The only role the ISBI plays in the Tanner–Vandeput Prize is to coordinate and award the Prize for each Quadrennial Congress. The International Burn Foundation has a Board of Directors and a Prize Committee which reviews applications and makes recommendations for award of each Prize.

The Prize Committee voted to award the first Tanner–Vandeput Prize, presented at the 7th International Congress held February 1986 in Melbourne, Australia to Dr Ian Alan Holder of the Shriners Burns Institute in Cincinnati, Ohio for his work on 'Infection by Pseudomonas Aeruginosa.' He was presented with a cash payment and a gold and diamond lapel pin signifying his achievement.